

**Listing of claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. **(Withdrawn/Currently amended)** A method for screening substances which are potential inhibitors of expression of bacterial T-box regulated genes, comprising the steps of:
  - a) incubating one or more assay mixtures to produce a readthrough mRNA product, wherein the assay mixtures comprise: a template DNA that comprises: (i) a bacterial promoter, (ii) a *glyQS* leader, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent magnesium cations ~~at a concentration equal to or higher than 30 mM~~; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the *glyQS* leader; bacterial RNA polymerase complex; and uncharged tRNA specific for a specifier sequence located in the *glyQS* leader; and
  - b) incubating a potential inhibitor substance with one or more assay mixtures to produce a readthrough mRNA product, wherein the assay mixtures comprise: a template DNA that comprises: (i) a bacterial promoter, (ii) a *glyQS* leader, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent magnesium cations ~~at a concentration of about 30 mM or higher~~; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the *glyQS* leader; bacterial RNA polymerase complex; and uncharged tRNA specific for a specifier sequence located in the *glyQS* leader;
  - c) comparing the fraction of total mRNA products corresponding to the read-through mRNA product produced in step a) with the fraction of total mRNA products corresponding to the read-through mRNA product produced in step b)  
wherein a lesser fraction of the read-through mRNA product produced in step b) in comparison with step a) indicates that said potential inhibitor substance inhibits transcriptional readthrough of said *glyQS* leader and therefore is an inhibitor of expression of bacterial T-box regulated genes.

2. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the divalent magnesium cation concentration is about 30 mM.
3. **(Withdrawn)** The method recited in claim 1 wherein the nucleoside triphosphates are selected from the group consisting of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, and any combination of one or more of these.
4. **(Withdrawn)** The method recited in claim 1 wherein the dinucleotides are selected from the group consisting of ApA, ApC, ApU, ApG, GpA, GpC, GpU, GpG, CpA, CpC, CpU, CpG, UpA, UpC, UpU, and UpG.
5. **(Withdrawn/Previously presented)** The method recited in claim 1 wherein the bacterial promoter is selected from the group consisting of a *B. subtilis* *glyQS* promoter and a *B. subtilis* *rpsD* promoter.
6. **(Withdrawn)** The method recited in claim 1 wherein the downstream polynucleotide of sufficient length for detection of a read-through mRNA product comprises a polynucleotide which is from about 30 to 150 nucleotide residues in length.
7. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the uncharged tRNA specific for a specifier sequence located in the *glyQS* leader is *B. subtilis* tRNA<sup>Gly</sup>.
8. **(Withdrawn/Previously presented)** The method recited in claim 1 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.
9. **(Withdrawn/Previously presented)** The method recited in claim 1 wherein the *glyQS* leader comprises a variant *glyQS* leader sequence which is a variant of a wild-type *glyQS* leader from a Gram positive bacterial strain, wherein the variant *glyQS* leader sequence comprises modifications to one or both *glyQS* leader specifier and antiterminator sequences as compared to the wild-type *glyQS* leader.
10. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the uncharged tRNA specific for a specifier sequence located in the *glyQS* leader is a variant of a wild-type tRNA in which either or both wild-type anticodon sequence, or wild-type discriminator sequence, are altered to complement the *glyQS* leader sequence.
11. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the *glyQS* leader comprises a variant *glyQS* leader sequence which is a variant of a wild-type *glyQS* leader from a Gram positive bacterial strain, wherein the variant *glyQS* leader sequence comprises modifications to one or both wild-type *glyQS* leader specifier and antiterminator sequences, and

wherein the uncharged tRNA specific for a specifier sequence located in the *glyQS* leader is a variant of a wild-type tRNA in which either or both wild-type anticodon sequence, or wild-type discriminator sequence are altered to complement the variant *glyQS* leader sequence.

12. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the assay mixtures comprises an *in vitro* halted-complex bacterial transcription assay systems.

13. **(Withdrawn/Previously presented)** A method for identifying inhibitors of expression of bacterial T-box regulated genes, comprising:

providing two or more *in vitro* halted-complex bacterial transcription assay systems which comprise a template DNA comprising: (i) a bacterial promoter, (ii) a polynucleotide comprising a portion of a leader from *B. subtilis* *glyQS* gene and including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; RNA polymerase, and uncharged *B. subtilis* tRNA<sup>Gly</sup>, wherein at least one or more of said assay systems comprises a test substance, and wherein at least one or more of said assay systems lacks a test substance, and comparing the fraction of total mRNA products corresponding to *B. subtilis* *glyQS* read-through mRNA produced in each of said assay systems, wherein a test substance is considered an inhibitor if it effects a lesser fraction of total mRNA products corresponding to the *B. subtilis* *glyQS* read-through mRNA produced in an assay system comprising said test substance as compared to an assay system lacking said test substance.

14. **(Withdrawn/Previously presented)** The method recited in claim 13 wherein the bacterial promoter is selected from the group consisting of a *B. subtilis* *glyQS* promoter and a *B. subtilis* *rpsD* promoter.

15. **(Withdrawn/Previously presented)** The method recited in claim 13 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.

16. **(Withdrawn/Previously presented)** The method recited in claim 13 wherein the polynucleotide comprising a portion of the leader from the *B. subtilis* *glyQS* gene comprises a variant *B. subtilis* *glyQS* leader sequence comprising modifications to one or both *B. subtilis* *glyQS* leader specifier and antiterminator sequences as compared to the wild-type *glyQS* leader, and

wherein the uncharged *B. subtilis* tRNA<sup>Gly</sup> is a variant of a wild-type *B. subtilis* tRNA<sup>Gly</sup> in which either or both wild-type anticodon sequence and wild-type discriminator sequence are altered to complement the variant *B. subtilis* *glyQS* leader sequence.

17. (Cancelled)

18. (Currently amended) A purified *in vitro* assay system for screening substances which are potential inhibitors of expression of bacterial T-box regulated genes, comprising:

a) one or more assay mixtures comprising: a template DNA that comprises: (i) a bacterial promoter, (ii) a *glyQS* leader, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent magnesium cations ~~at a concentration of about 30 mM or higher~~; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the *glyQS* leader; bacterial RNA polymerase complex; and uncharged tRNA specific for a specifier sequence located in the *glyQS* leader; and

b) one or more assay mixtures comprising: a potential inhibitor substance; a template DNA that comprises: (i) a bacterial promoter, (ii) a *glyQS* leader, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent magnesium cations ~~at a concentration of about 3 mM or higher~~; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the *glyQS* leader; bacterial RNA polymerase complex; and uncharged tRNA specific for a specifier sequence located in the *glyQS* leader.

19. (Previously presented) The assay system recited in claim 18 wherein the divalent magnesium cation concentration is about 30 mM.

20. (Original) The assay system recited in claim 18 wherein the nucleoside triphosphates are selected from the group consisting of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, and any combination of one or more of these.

21. (Original) The assay system recited in claim 18 wherein the dinucleotides are selected from the group consisting of ApA, ApC, ApU, ApG, GpA, GpC, GpU, GpG, CpA, CpC, CpU, CpG, UpA, UpC, UpU, and UpG.

22. (Previously presented) The assay system recited in claim 18 wherein the bacterial promoter is selected from the group consisting of a *B. subtilis* *glyQS* promoter and a *B. subtilis* *rpsD* promoter.

23. **(Original)** The assay system recited in claim 18 wherein the downstream polynucleotide of sufficient length for detection of a read-through mRNA product comprises a polynucleotide which is from about 30 to 150 nucleotide residues in length.
24. **(Currently amended)** The assay system recited in claim 18 wherein the uncharged tRNA specific for a specifier sequence located in the *glyQS* leader is *B. subtilis* tRNA<sup>Gly</sup>.
25. **(Previously presented)** The assay system recited in claim 18 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.
26. **(Previously presented)** The assay system recited in claim 18 wherein the *glyQS* leader comprises a variant *glyQS* leader sequence which is a variant of a wild-type *glyQS* leader from a Gram positive bacterial strain, wherein the variant *glyQS* leader sequence comprises modifications to one or both *glyQS* leader specifier and antiterminator sequences as compared to the wild-type *glyQS* leader.
27. **(Currently amended)** The assay system recited in claim 18 wherein the uncharged tRNA specific for a specifier located in the *glyQS* leader is a variant of a wild-type tRNA in which either or both wild-type anticodon sequence, or wild-type discriminator sequence, are altered to complement the *glyQS* leader sequence.
28. **(Currently amended)** The assay system recited in claim 18 wherein the leader comprises a variant *glyQS* leader sequence which is a variant of a wild-type *glyQS* leader from a Gram positive bacterial strain, wherein the variant *glyQS* leader sequence comprises modifications to one or both *glyQS* leader specifier antiterminator sequences as compared to the wild-type *glyQS* leader, and wherein the uncharged tRNA specific for a specifier sequence located in the *glyQS* leader is a variant of a wild-type tRNA in which either or both wild-type anticodon sequence, or wild-type discriminator sequence are altered to complement the variant *glyQS* leader sequence.
29. **(Currently amended)** The assay system recited in claim 18 wherein the assay comprises an *in vitro* halted-complex bacterial transcription assay system.
30. **(Currently amended)** A purified *in vitro* assay system for identifying inhibitors of expression of bacterial T-box regulated genes, comprising:

two or more *in vitro* halted-complex bacterial transcription assay systems which comprise a template DNA comprising: (i) a bacterial promoter, (ii) a polynucleotide comprising a portion of a leader from *B. subtilis* *glyQS* gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; RNA

polymerase, and uncharged *B. subtilis* tRNA<sup>Gly</sup>, wherein at least one or more of said assay systems comprises a test substance, and wherein at least one or more of said assay systems lacks a test substance.

31. **(Currently amended)** The assay system recited in claim 30 wherein the bacterial promoter is selected from the group consisting of a *B. subtilis* *glyQS* promoter and a *B. subtilis* *rpsD* promoter.

32. **(Currently amended)** The assay system recited in claim 30 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.

33. **(Currently amended)** The assay system recited in claim 30 wherein the polynucleotide comprising a portion of the leader from the *B. subtilis* *glyQS* gene comprises a variant *B. subtilis* *glyQS* leader sequence comprising modifications to one or both *B. subtilis* *glyQS* leader specifier and antiterminator sequences as compared to the wild-type *glyQS* leader, and wherein the uncharged *B. subtilis* tRNA<sup>Gly</sup> is a variant of a wild-type *B. subtilis* tRNA<sup>Gly</sup> in which either or both wild-type anticodon sequence and wild-type discriminator sequence are altered to complement the variant *B. subtilis* *glyQS* leader sequence.

34. **(Cancelled)**

35. **(Cancelled)**